

Listing of Claims

What is claimed is:

1. (Currently Amended) An isolated aggrecan peptide fragment containing comprising a specific ADMP-susceptible cleavage site.
2. (Currently Amended) A peptide fragment of claim 1 wherein the peptide has a linking-moiety.
3. (Currently Amended) An isolated aggrecan peptide fragment comprising consisting of a sequence of amino acids 1-40 of SEQ ID NO:1.
4. (Currently Amended) An isolated aggrecan peptide fragment comprising a sequence of amino acids that is at least 80% identical to the sequence consisting of amino acids 1-40 of SEQ ID NO:1.
5. (Currently Amended) An isolated aggrecan peptide fragment of comprising consisting of a sequence of amino acids 1-40 of SEQ ID NO:2.
6. (Currently Amended) [(13)] An isolated aggrecan peptide fragment comprising a sequence consisting of amino acids 1-40 of SEQ ID NO:3.
7. (Currently Amended) [(14)] An isolated aggrecan peptide fragment comprising a sequence that is at least 80% identical to the sequence consisting of amino acids 1-40 of SEQ ID NO:3.
8. (Original) A peptide of claims 1, 2, 3, 4, 5, 6 or 7 wherein the peptide is biotinylated.
9. (Original) A peptide of claim 2 wherein the linking-moiety is a biotinylated lysine.

10. (Original) A peptide of claim 2 wherein the linking-moiety contains a chromophore.
11. (Original) A peptide of claim 2 wherein the peptide has a C-terminal linking-moiety.
12. (Original) A peptide of claim 2 wherein the peptide has a C-terminal linking-moiety that is a biotinylated lysine.
13. (Original) A peptide of claim 2 wherein the peptide has an N-terminal linking-moiety.
14. (Original) A peptide of claim 2 wherein the peptide has an N-terminal linking-moiety that is a biotinylated lysine.
15. (Currently Amended) An isolated proteolytic cleavage product, of the isolated peptide fragment of claim 1, comprising the amino acids from the N-terminus through P1 of the ADMP-susceptible cleavage bond.
16. (Currently Amended) An isolated proteolytic cleavage product, of the isolated peptide of claim 1, comprising the amino acids from P1' of the ADMP-susceptible cleavage bond through the C-terminus.
17. (Currently Amended) A proteolytic cleavage product of claims 15 or 16 wherein the peptide is biotinylated.
18. (Currently Amended) A proteolytic cleavage product peptide of claim 15 wherein the peptide has an N-terminal linking-moiety.
19. (Currently Amended) A proteolytic cleavage product peptide of claim 16 wherein the peptide has a C-terminal linking-moiety.

20. (Currently Amended) A proteolytic cleavage product peptide of claim 18 wherein the linking-moiety is a biotinylated lysine.

21. (Currently Amended) A proteolytic cleavage product peptide of claim 19 wherein the linking-moiety is a biotinylated lysine.

22. (Currently Amended) A proteolytic cleavage product peptide of claim 18 wherein the linking-moiety contains a chromophore.

23. (Currently Amended) A proteolytic cleavage product peptide of claim 19 wherein the linking-moiety contains a chromophore.

24. (Currently Amended) An isolated, C-terminal biotinylated, aggrecan peptide fragment comprising a sequence of amino acids 20-40 of claim 3, wherein an additional biotinylated lysine is attached to the C terminus via a peptide bond, comprising a sequence of amino acids of SEQ ID NO:5.

25. (Currently Amended) An isolated, N-terminal biotinylated, aggrecan peptide fragment comprising a sequence of amino acids 1-20 of claim 3, wherein an additional biotinylated lysine is attached to the N terminus via a peptide bond, comprising a sequence of amino acids of SEQ ID NO:6.

26. (Withdrawn) A method for the determination of the presence of aggrecan-degrading metalloprotease activity comprising: (a) binding an ADMP substrate peptide of claim 1 to a streptavidin-coated microtiter plate; (b) rinsing the microtiter plate with assay buffer; (c) incubating the microtiter plate with an ADMP-containing sample; (d) rinsing the microtiter plate; (e) incubating the microtiter plate with a neoepitope antibody solution; (f) rinsing the microtiter plate; (g) incubating microtiter plates with secondary-

detection antibody solution; (h) incubating the microtiter plate with an appropriate substrate solution; (i) quenching the reaction; (j) reading the optical density;

27. (Withdrawn) The method of claim 26, wherein said ADMP peptide substrate comprises a covalently-linked linking-moiety.

28. (Withdrawn) A method for the determination of ADMP activity by quantifying the appearance of a product peptide comprising: (a) incubating an ADMP substrate peptide of claim 1 with assay buffer and ADMP-containing sample; (b) quenching the reaction; (c) injecting a portion of the reaction mixture onto a reverse-phase HPLC column; (d) eluting the peptide with an organic solvent; (e) reading the absorbance; (f) determining the quantity based on a standard curve.

29. (Withdrawn) A method for assaying compounds for activity against an ADMP comprising: (a) providing an ADMP and an ADMP substrate; (b) contacting said ADMP with a candidate inhibitory compound in the presence of said ADMP; and (c) measuring the inhibition of the ADMP activity.

30. (Withdrawn) A method for assaying compounds according to claim 29 wherein the ADMP activity is monitored according to claim 26 or 28.

31. (Original) A peptide of claim 3, 4, or 5 wherein the P1 amino acid residue, Glu, of the ADMP-sensitive Glu³⁷³-Ala³⁷⁴ bond, is esterified.

32. (Original) A peptide of claim 3, 4, or 5 wherein the P1 amino acid residue, Glu, of the ADMP-sensitive Glu³⁷³-Ala³⁷⁴ bond, is replaced with a Gln amino acid residue.

33. (Withdrawn) An assay for detecting ADMP activity which comprises: (a) incubating a sample containing soluble ADMPs or aggrecanase activity with an aggrecan substrate; and (b) monitoring production of aggrecan fragments produced by specific cleavage at an ADMP-susceptible site using a neoepitope antibody to the new N-terminus or the new C-terminus generated by specific ADMP-mediated cleavage by the Problot assay comprising: (1) incubate a polyvinyl-denedifluoride (PVDF) cationically charged membrane, secured in a welled filtration plate, with a sample containing ADMP-degraded aggrecan; (2) wash any unbound aggrecan from the filtration plate; (3) couple any unreacted cationic sites on the PVDF membrane with a solution of bovine serum albumin (BSA); (4) wash any unbound BSA from the filtration plate; (5) remove glycosaminoglycan side chains from the bound aggrecan with deglycosylation enzymes, wash membrane; (6) incubate PVDF membrane with a neoepitope antibody to fragments generated by cleavage at an ADMP-sensitive site, wash membrane; (7) incubate PVDF membrane with secondary detection antibody, wash membrane; (8) incubate PVDF membrane with detection substrate; (9) drain solution into welled plate, obtain absorbance readings on individual samples; compare values to those obtained for standard curve.

34. (Withdrawn) A method for assaying compounds according to claim 29 wherein ADMP activity is monitored according to claim 33.

35. (Withdrawn) An assay according to claim 33 wherein the tissue or cell source of ADMPs is cartilage or chondrocytes.

36. (Withdrawn) An assay according to claim 33 or 34 wherein the aggrecan substrate is native aggrecan isolated from human or animal tissue.

37. (Withdrawn) An assay according to claim 33 or 34 wherein the aggrecan substrate is a recombinant aggrecan molecule or recombinant portion of the aggrecan molecule containing an aggrecanase-sensitive cleavage site.

38. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E³⁷³⁻³⁷⁴A bond.

39. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant aggrecan fragment contains the E¹⁵⁴⁵⁻¹⁵⁴⁶G bond.

40. (Withdrawn) An assay according to claim 33 or 34 wherein the portion of the aggrecan molecule contains the E¹⁷¹⁴⁻¹⁷¹⁵G bond.

41. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E¹⁸¹⁹⁻¹⁸²⁰A bond.

42. (Withdrawn) An assay according to claim 33 or 34 wherein the recombinant portion of the aggrecan molecule contains the E¹⁹¹⁹⁻¹⁹²⁰L bond.

43. (Withdrawn) A method according to claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E373 -A374 bond.

44. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody is the BC-3 monoclonal antibody.

45. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1545-G1546 bond.

46. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1714-G1715 bond.

47. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1819-A1820 bond.

48. (Withdrawn) A method of any of claims 26, 30, 33, or 34 wherein the neoepitope antibody recognizes the new N-terminus or new C-terminus generated by cleavage at the E1919-L1920 bond.

49. (Withdrawn) A method of use of the assay in claim 33 for detecting ADMP-generated aggrecan fragments in culture media from tissue or cell cultures stimulated to induce aggrecanase-mediated degradation.

50. (Withdrawn) A method of use of the assay in claim 33 for detecting aggrecanase-generated aggrecan fragments in biological fluids, tissue extracts or homogenates, serum or urine from patients with aggrecanase-associated diseases.

51. (Withdrawn) A method for diagnosing arthritic diseases in a mammal by monitoring ADMP-generated aggrecan fragments according to claims 33.

52. (Withdrawn) A method for diagnosing a disease in a mammal characterized by overproduction or up-regulated production of an ADMP by monitoring fragments generated at an ADMP-sensitive site according to claims 33.

53. (New) An isolated aggrecan peptide fragment comprising a specific ADMP-susceptible cleavage site wherein said cleavage site is the bond between the amino acid pairs selected from the group consisting of Glu³⁷³-Ala³⁷⁴, E¹⁵⁴⁵-G¹⁵⁴⁶, E¹⁷¹⁴-G¹⁷¹⁵, E¹⁸¹⁹-A¹⁸²⁰, and E¹⁹¹⁹-L¹⁹²⁰, wherein said numbering corresponds to the numbering of human aggrecan protein.